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ONE KIND OF INTRAMEMBRANE PARTICLE IS WATER SOLUBLE

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Mammalian urothelial cell membranes contain paracrystalline plaques 0.3–0.5 μm Diam consisting of particles 120 Å Diam in a hexagonal lattice with a constant of 160 Å. Each particle in negative stain consists of a ring of twelve subunits as determined by image analysis (1, 2). These surround a central depression or pore. The membrane between the plaques is smooth and referred to as the "hinge" membrane. In thin transverse sections the hinge membrane appears as a unit membrane ~ 75 Å thick, whereas the plaque membrane is ~ 130 Å thick. In freeze-fracture-etch (FFE) preparations, the external fracture (EF) faces display particles about the same size as the external ones, also in a hexagonal array with a lattice constant of 160 Å. The PF faces have corresponding plaque regions with the same lattice constant, but there are no pits to correspond to the particles in the EF face. Instead, the PF pattern consists of repeating domains, each made of a ring of metal surrounding a slight depression with a spot of metal in the center. The PF pattern is easily destroyed by etching but the EF pattern is very resistant. We have recently published (3) a study focused mainly on the EF faces in which we presented evidence that the EF particles are artifacts. We believe our findings indicate that the particles are plastic deformation and decoration artifacts.

The isolated membranes were deposited on a glass coverslip, covered with a copper sheet and fractured under liquid nitrogen (LN_2). We replicated fractured membranes on the glass surface and measured the height of the EF particles above the glass surface. It was greater than the total thickness of the membrane and thus the particles

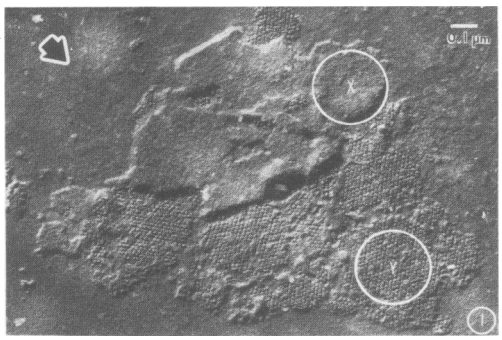
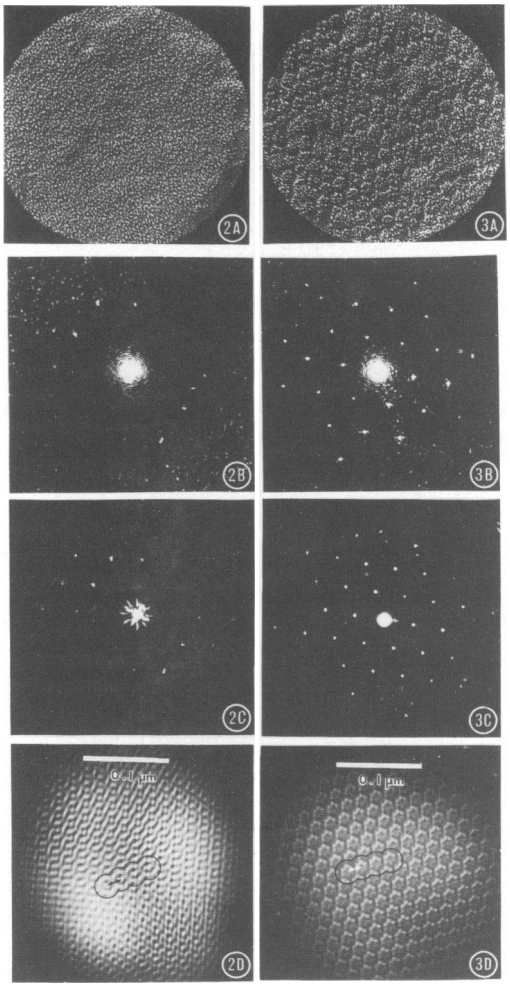


FIGURE 1 FFE preparation of mammalian urothelial membranes deposited on glass. Two superimposed membranes are shown, both of which are fractured to reveal parts of their EF faces. Magnification as indicated.



FIGURES 2 AND 3 FFE preparation of mammaliam urothelial membranes deposited on glass. 2 *A* and 3 *A* are enlargements of areas *X* and *Y*, respectively, from Fig. 1. 2 *B–D* and 3 *B–D* are areas *X* and *Y* from Fig. 1 analyzed by image filtration. 2 *A* and 3 *A* are at the same magnification as 2 *D* and 3 *D*.

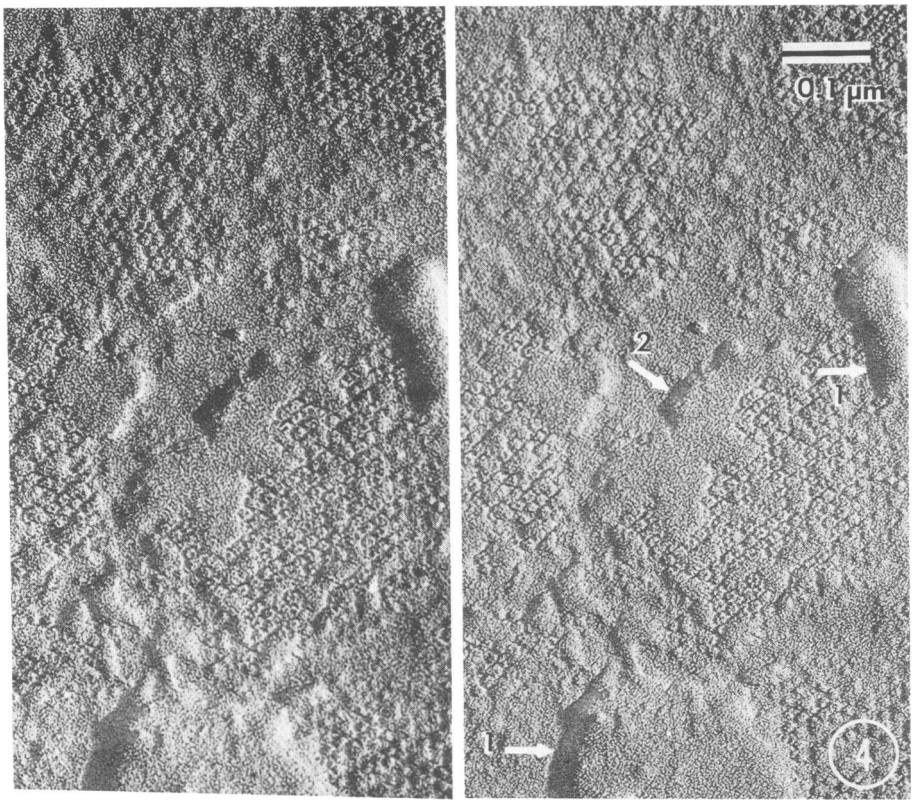


FIGURE 4 Stereo pair of micrographs showing EF faces of a bladder membrane prepared as in Fig. 1. Note that the particles are covered by smooth areas that are elevated slightly above the level of the particle with respect to the glass. These smooth areas correspond to area *X* seen in Fig. 1. Magnification as indicated.

would of necessity protrude through the PF half of the membrane if the particles were real. No pits or holes have ever been found in the PF face and so the particles are artifacts.

RESULTS AND DISCUSSION

We now report on further studies of this membrane extending the same technique. However, in this case the membranes, after being fractured under liquid nitrogen, were thawed, washed with water, air dried, returned to liquid nitrogen, and replicated at low temperature. We were pleased to find that this procedure still left identifiable membranes on the glass. Fig. 1 shows an area in which a particulate lattice can be made out in the area marked *Y*, enlarged in Fig. 3 *A*. The particles here are in a hexagonal lattice with a lattice constant of 160 Å. We also observed other areas in the preparation in which smooth fracture faces were seen such as those in area *X* in Fig. 1, enlarged in Fig. 2 *A*. An image analysis of the area in Fig. 2 *A* is presented in Fig. 2 *B–D*. The diffraction pattern from the area is seen in *B* directly and in *C* through a mask used for image filtration. *D* shows the filtered image. This image is almost identical to the filtered image of the smooth EF faces described in detail in our previous paper (Fig. 4 *D* in reference 3). Fig. 3 *B–D* presents a similar image analysis of area *Y*. The filtered image in *D* is almost identical to the filtered image of the external particle lattice analyzed in our previous paper (Fig. 3 *D* in reference 3). We have found that there are many places in the preparation in which the particle lattice is covered by material like that in area *X* in Fig. 1. Several such areas are shown in the stereo pair in Fig. 4 *A* and *B*. It is clear when observed in stereo that the smooth areas are slightly higher above the glass than the particles. We have measured the heights of these smooth areas above the glass

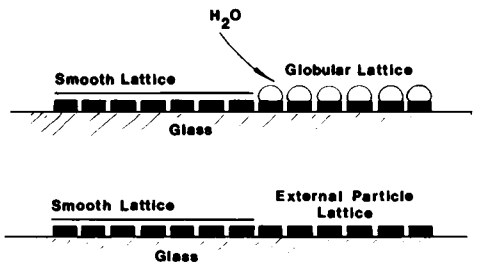


FIGURE 5 Imaginery cross sections through a bladder membrane attached to a glass surface, frozen, and fractured. The external protein particles are shown as black rectangular blocks. The diagram illustrates the effect of washing the fracture faces with water after the frozen half membranes have been thawed. The globular particles are washed away by the water exposng the back surface of the protein lattice that normally faces the bilayer. The smooth lattice is not affected by the water.

as well as the heights of the particles and the heights of other smooth areas which do not give a diffraction pattern (1 in Fig. 4).

In our previous study we found that smooth EF pattern areas occur in preparations fractured under LN_2 in alternation with the globular particle areas. We presented reasons in the previous paper for believing that these smooth areas represented the true EF face with the globular particle lattice being an artifactural structure derived from the smooth lattice. We interpret our present findings in the following way. The heights of the particles above the glass in areas like *Y* in Fig. 1 are consistent with the particulate lattice's being the surface particle lattice seen from the back side, i.e., from the side normally covered by the bilayer portion of the membrane. The optical diffraction analysis supports this interpretation in that the filtered images are almost identical to those given by the particle lattice replicated from the external side. The smooth areas that give diffraction patterns (2 in Fig. 4), which lie only slightly higher above the glass than the particle lattice, represent the smooth lattice that we identified in our previous paper as the true external fracture face. We interpret this as a lipid-carbon chain surface. The smooth surfaces lying at a greater height above the glass (1 in Fig. 4), which do not give diffraction patterns, we interpret as the protoplasmic surfaces of unfractured membranes. In the case of Fig. 1, we are dealing with two membranes oriented with their external surfaces toward the glass but superimposed on one another. In the case of the one containing area *Y*, the facture plane originally produced mainly the globular lattice as described in reference (3). There are a few identifiable limited areas of the smooth lattice obscuring the particles. The more elevated membrane superimposed on this one fractured almost completely without forming the globular lattice. Instead it was, after fracture, almost entirely a smooth lattice. This smooth lattice, according to our interpretation, is a lipid-carbon chain surface and hence hydrophobic. In contrast, the previously described (3) globular particle lattice we interpret as being produced by plastic deformation in the lipid bilayer and subsequent decoration. We believe that the large globular particles would have been seen in the present preparation in area *Y* if it had not been washed with water after fracture. Evidently, the structures responsible for the globular particles in unwashed preparations were washed away by water after thawing. This probably means that the seed structure in forming the globular particles is a lipid micelle with polar groups oriented outward produced from the external lipid monolayer by the fracturing process. The shock of fracturing evidently detaches these lipid molecules from the surface protein particles so that they can be washed away. The diagram illustrates the washing procedure and its effect on the fracture faces (Fig. 5).

This work was supported by grant 1 RO1 GM28244 from the National Institutes of Health.

Received for publication 8 May 1981.

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LIPID-DEPENDENT STRUCTURAL CHANGES OF AN AMPHOMORPHIC MEMBRANE PROTEIN

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The coat protein from the filamentous phages fd, f1, and M13 is easily obtained and readily associates with lipids. These protein lipid complexes have been used frequently as model membranes for biophysical studies (1–8). Here we report that the fd coat protein undergoes an $\alpha \rightleftharpoons \beta$ conformational transition that depends on the lipid:protein ratio and on the nature of the lipid tail groups. "Amphomorphic" is proposed as a term for proteins that undergo environmentally mediated $\alpha \rightleftharpoons \beta$ transitions.

RESULTS AND DISCUSSION

Raman spectroscopy (6, 8) and circular dichroism (1, 2) were used to distinguish "50%α" from "50%β". By curve fitting the circular dichroism (CD) and Raman spectra we find no α-helix in the "50% β" structure and little, if any, β structure in the "50% α".

Tanford and co-workers report "50%α" to be the favored conformation in amphiphiles and the β-polymer to